

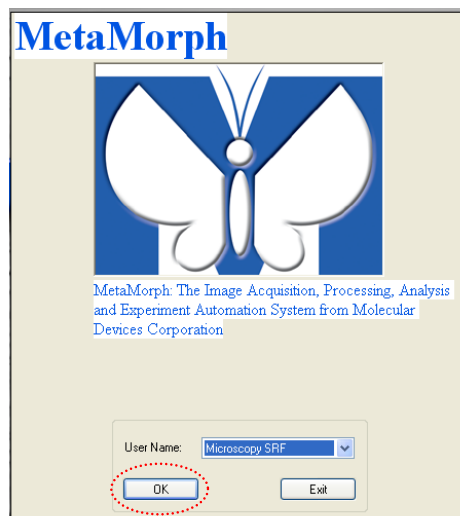
Spinning Disk Protocol

1) System Startup

- ☞ Please note our sign-up policy. You must inform the facility at least 24 hours beforehand if you can't come; otherwise, you will receive a charge for unused time. The facility will allow for extenuating circumstances (cells dying, sick day, etc.) if you inform us in a timely fashion.
- ☞ **Follow each step** of the startup poster.
- ☞ **Log into** the computer with your user account.



- ☞ **Open MetaMorph.**
- ☞ On the following screen, **ensure Microscopy SRF is selected as the User Name**, and click **OK**.

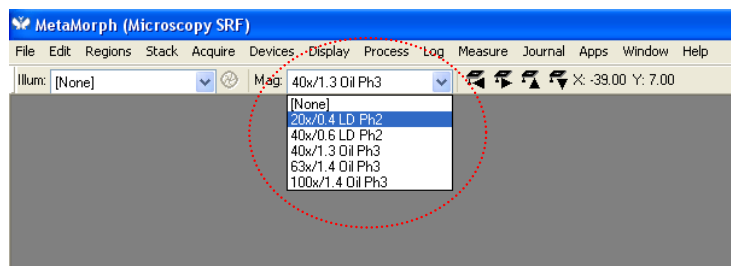


2) Lens Cleaning

- ☞ Please **clean all of the lenses** (used and unused) **before and after** your session. Refer to the lens cleaning poster if you need any help recalling the rules and steps.

3) Microscope control

- ☞ First, use the magnification control selection within the MetaMorph software to switch between your microscope lenses.



☞ Here are the lens immersion classifications of the options in the dropdown menu:


- Long distance: 20x, 40x
- Oil Immersion: 40x, 63x, 100x
- Please note the following points:
 - First, since the microscope recalls the focal position between each lens, properly set your focal position with a lower power lens first before loading your slides and before using a higher power lens. For example, start with 20x lens, then proceed to oil immersion as necessary.
 - Secondly, when switching between lenses that use different immersions, take the appropriate action needed to move to the next lens: apply oil when switching from a dry lens to oil lens, and remove oil when switching from an oil lens to a dry lens.
- Before you load your slide, **select the 20x lens.**

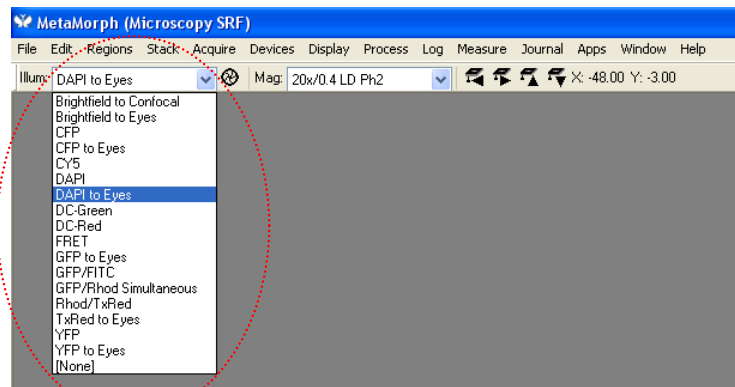
☞ **Load your slide** upside down and take care for the following issues:

- **Select and inspect each slide or dish.**
 - If it is dirty, gently clean with a KimWipe and/or cotton swab. You should do this with all your samples before you come.
 - Carefully rest the slide or dish onto the stage, and adjust the stage adapter to fit accordingly.

☞ Use the stage movement control joystick to position your specimen under the lens.

☞ Use the illumination control selection within the MetaMorph software to view and focus your sample. Be sure to

select the settings which indicate “to Eyes.” Click the shutter icon  to open and close the light shutter.



☞ Focus on your sample and center your region of interest.

☞ **Check all of your fluorophores** in your specimen. You need to have an idea of what to expect before you start your scan.

☞ Press the shutter icon again to close the light shutter to prevent photobleaching of your sample.

4) *Setting up your multidimensional experiment*

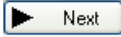
☞ From the Acquisition taskbar, select the **Primary Camera (left)** to activate the primary camera.

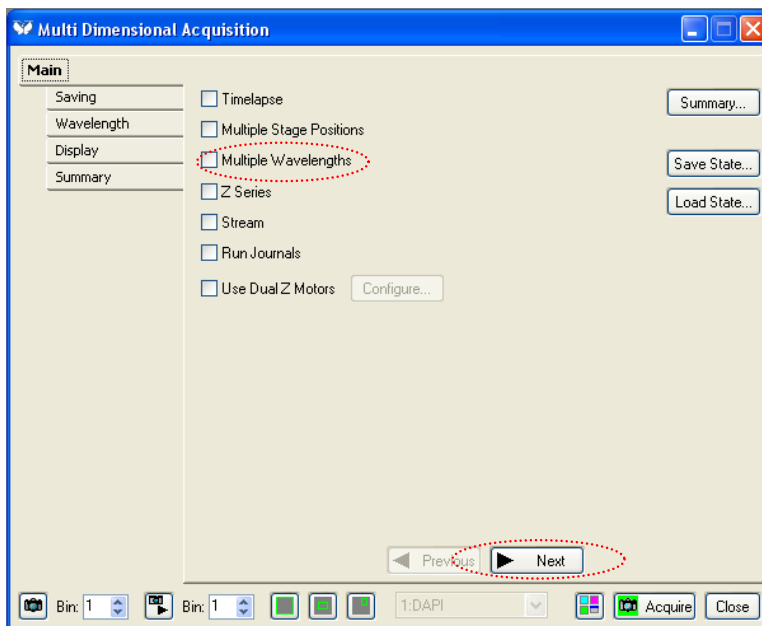
☞ From the Acquisition taskbar, **click Select the Zeiss Focus** to activate the Zeiss focus.




☞ Within the Acquisition taskbar, **open the Multi Dimensional Acquisition** menu.

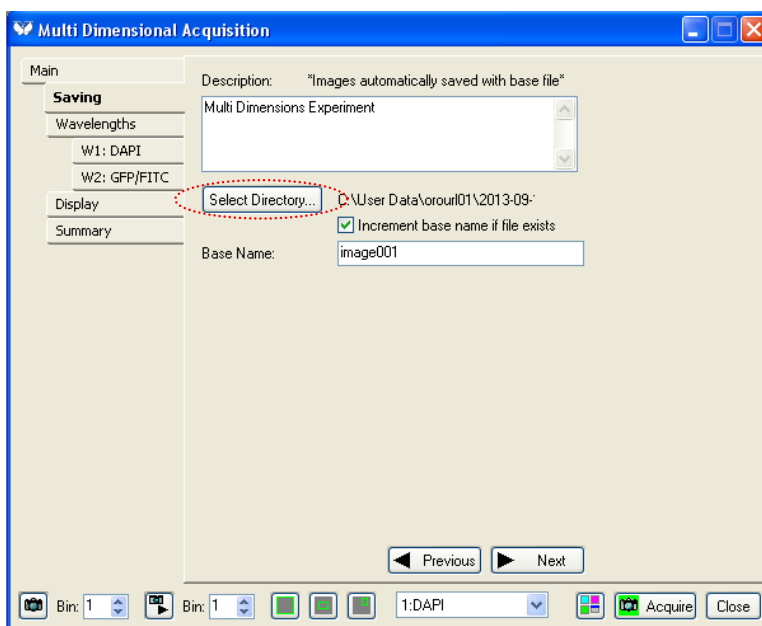
☞ The Multi Dimensional Acquisition (MDA) menu will open.


☞ In the Main tab, select Multiple Wavelengths. Progress to the next tab by clicking .

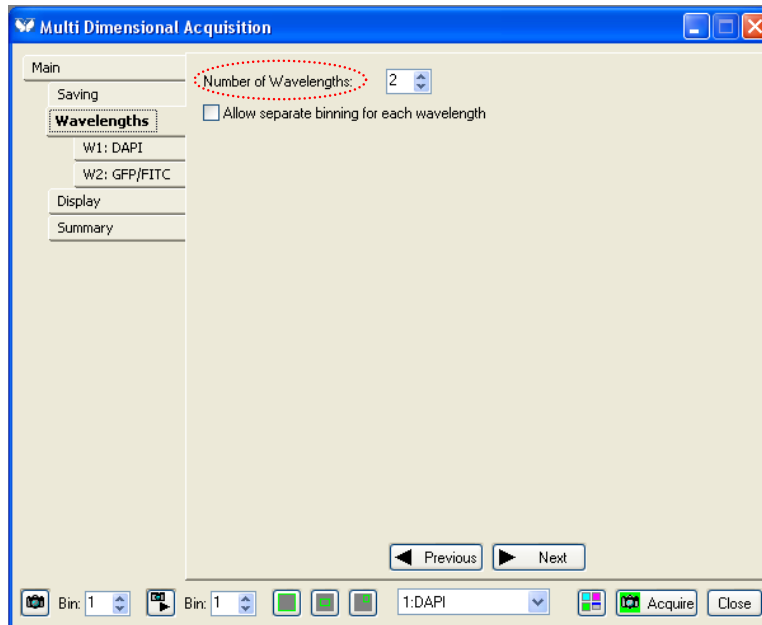


☞ In the Save tab, click  and **select your User Data folder** from the C:\ drive.

☞ In the “Base Name:” entry, designate a name for your images. You may end this name with a number (ie “image001”) and check **Increment base name if file exists** . This way, the software will automatically increment this number for each image acquired. Progress to the next tab by clicking .



- ☞ In the Wavelengths tab, select the number of wavelengths you wish to acquire. For example, a sample stained with DAPI, Alexa 488, and Alexa 555 would have 3 wavelengths. Progress to the next tab by clicking .

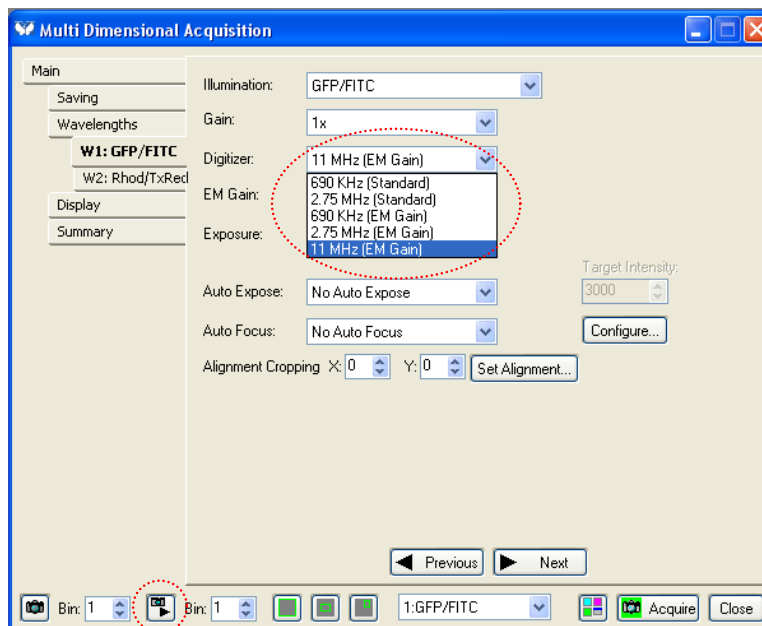



- ☞ In the following wavelength tabs (W1, W2...), select the appropriate illumination setting.

- **Note:** Be careful not to select a “to Eyes” setting!

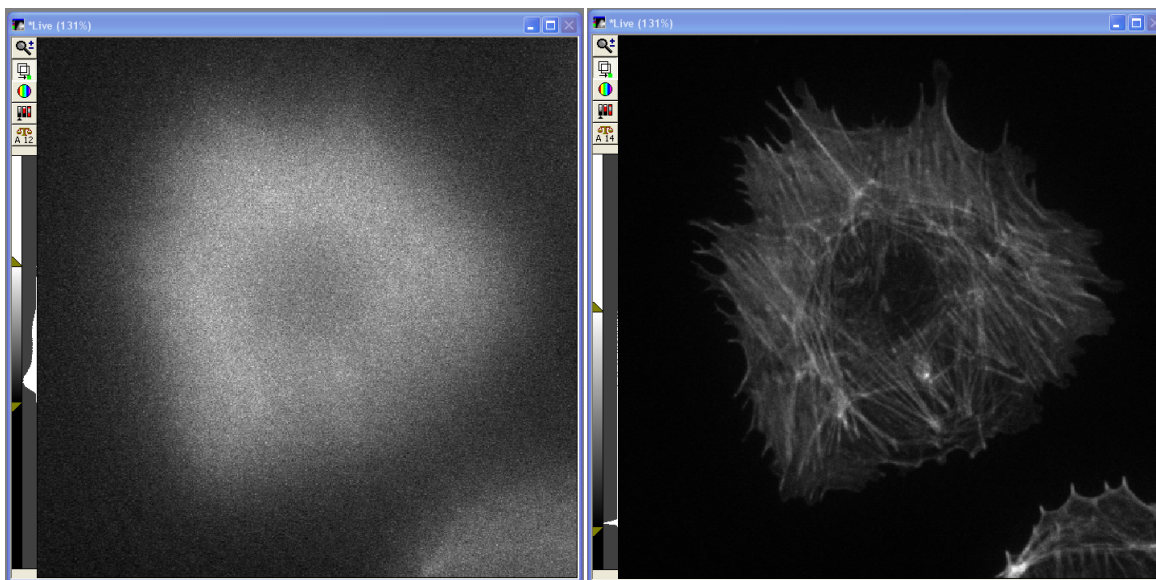
- ☞ Select a digitizer setting. Be sure all wavelengths have the same digitizer setting selected.

- 690 KHz (Standard): slowest camera setting, no camera gain
- 2.75 MHz (Standard): faster camera setting, no camera gain
- 690 KHz (EM Gain): slowest camera setting, with camera gain
- 2.75 MHz (EM Gain): faster camera setting, with camera gain
- 11 MHz (EM Gain) : fastest camera setting, with camera gain




Click  to **show the live view** of the selected wavelength.



The sample will be out of focus. Carefully adjust the focus until the image is sharp.

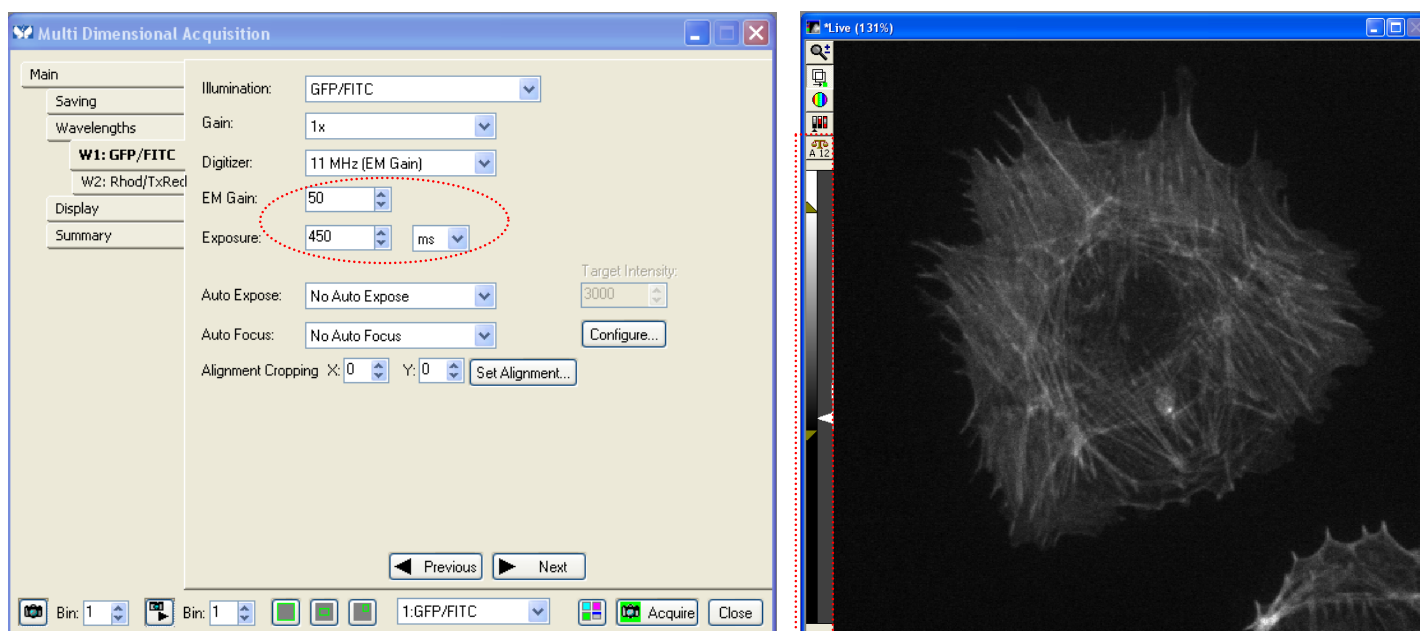




BEFORE

AFTER

The bit depth of the image and a histogram are visible along the left side of the live image. The bit depth is indicated by the number in the  icon. Since the cameras on the Spinning Disk system are 12-bit cameras, the images should likewise be in 12 bits.

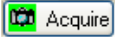
- If this number indicates a bit depth higher than 12 bits, reduce the exposure time or EM Gain settings until the icon reads .
- If this number indicates a bit depth lower than 12 bits, increase the exposure time or EM Gain settings until the icon reads .




Click  again to **turn the live view off**. Progress to the next tab by clicking .

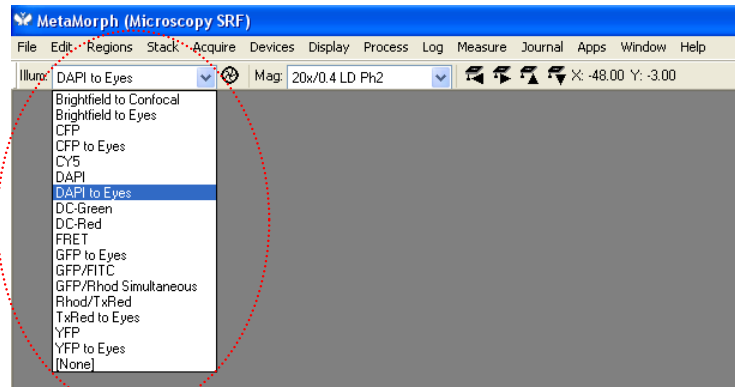
☞ Repeat the past 6 steps for each wavelength in the acquisition

- **Note: Be sure all wavelengths have the same digitizer setting selected!**

☞ Once all wavelengths have been set up, click . All channels will be acquired and automatically written to the drive designated in “Save.”

5) *Returning to the microscope*

☞ To return to the microscope to find another field, use the illumination control selection within the MetaMorph software to view and focus your sample. Be sure to select the settings which indicate “to Eyes.” Click the shutter icon  to open and close the light shutter.



☞ Focus on your sample and center your region of interest.

☞ You also can perform the following operations after returning to the microscope:

☞ *Selecting higher magnification*

- If you need to switch to the 40x, 63x, or 100x oil immersion lens, first select the desired lens in the software.
- Lower the lenses by pressing the lower Z button above the focus knob on the right base of the microscope, pictured below.



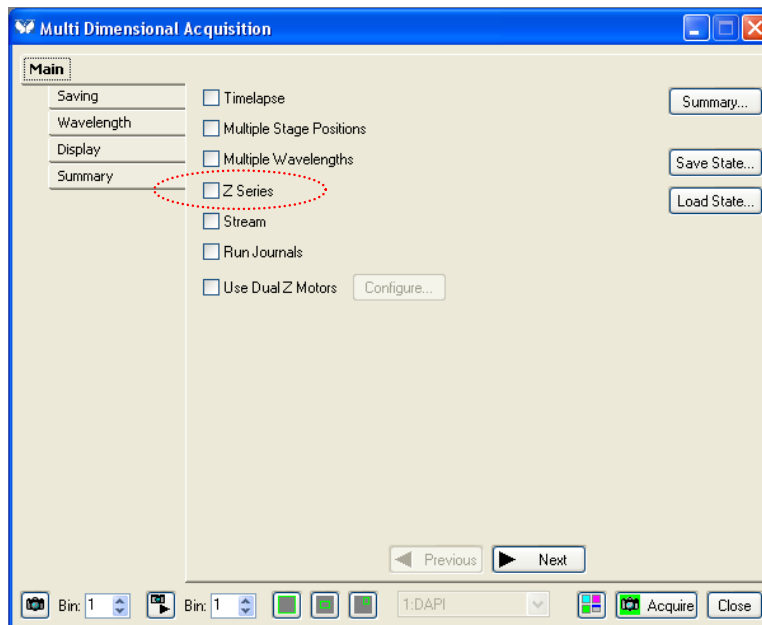
- Slightly turn the lenses to gain access to the current lens; if it's still unreachable, remove the specimen.
- Apply one drop of oil to the center of the lens and return it or specimen to original position.
- Press the raise Z button. You should see oil contact between the lens and coverslip.
- Focus & center your specimen. Please remember to use the fine focus control!

☞ *Switching slides*

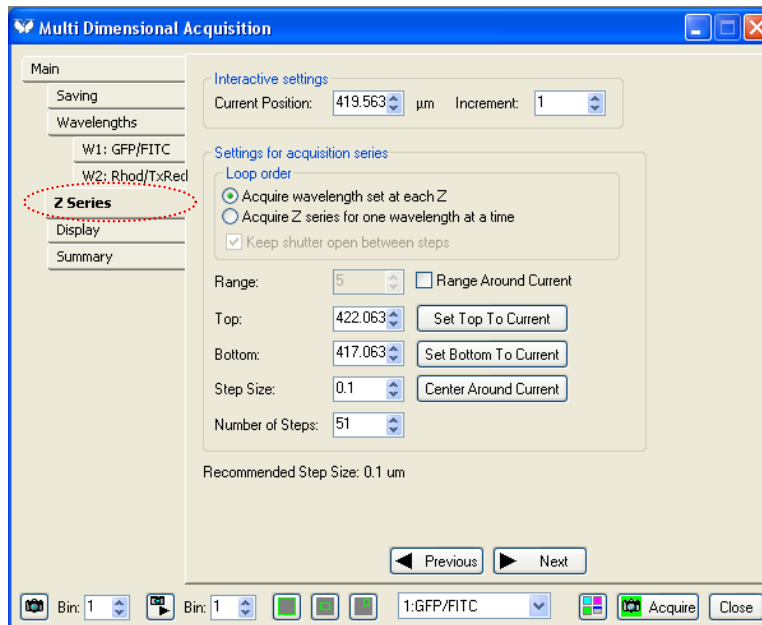
- Lower the lenses by pressing lower Z button above the focus knob on the right base of the microscope, or by using the coarse focus.
- Remove your specimen.
- Switch the lens to the 20x or 40x dry lens with the software.
- Load the new specimen.
- Press the raise Z button.
- Focus and center your specimen.
- Proceed with imaging or switching to a higher power lens.


6) *Setting up your Z-stack*


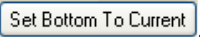
☞ In the Main tab, select Z Series.




☞ Select the Z Series tab within the MDA.



☞ Click  to set your stack limits:

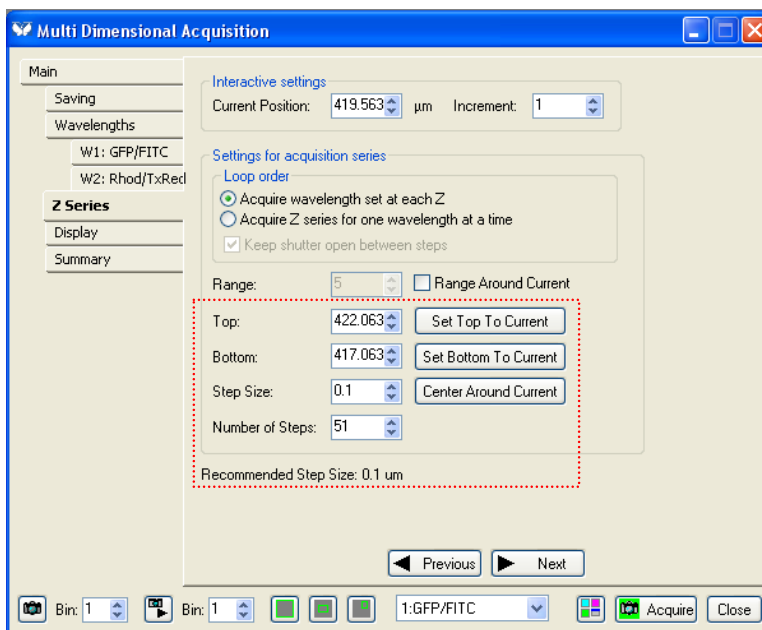
- Use the focus knob to set the focal plane towards the top of your specimen, and click .
- Use the focus knob to set the focal plane towards bottom of your specimen and click .

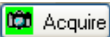
☞ Click  again to turn the live view off.

☞ Note the step size and number of steps. The software displays the **Recommended Step Size:** below the Z Series settings.

This recommended step size is the optimal size for post processing techniques like deconvolution or 3D reconstruction, but you may not need that many slices or to have such small Z slice intervals.

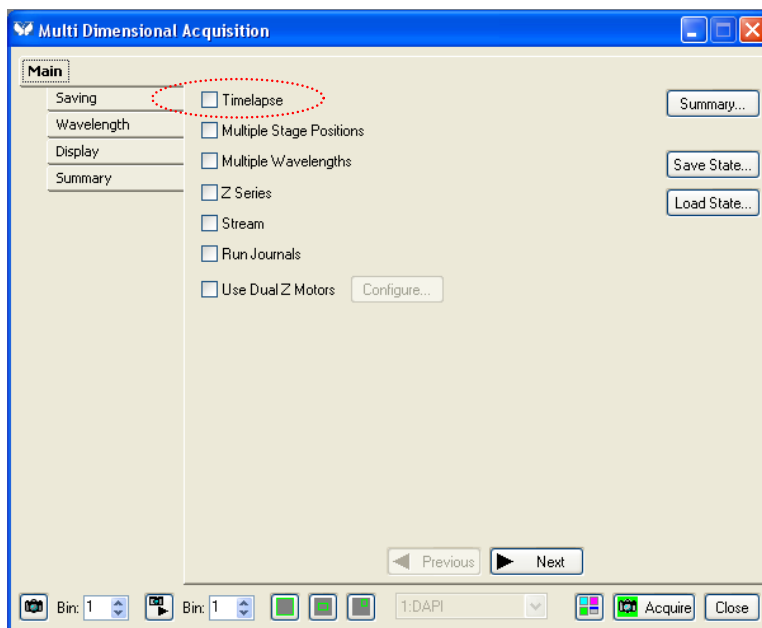
☞ Input either your desired number of steps or step size.



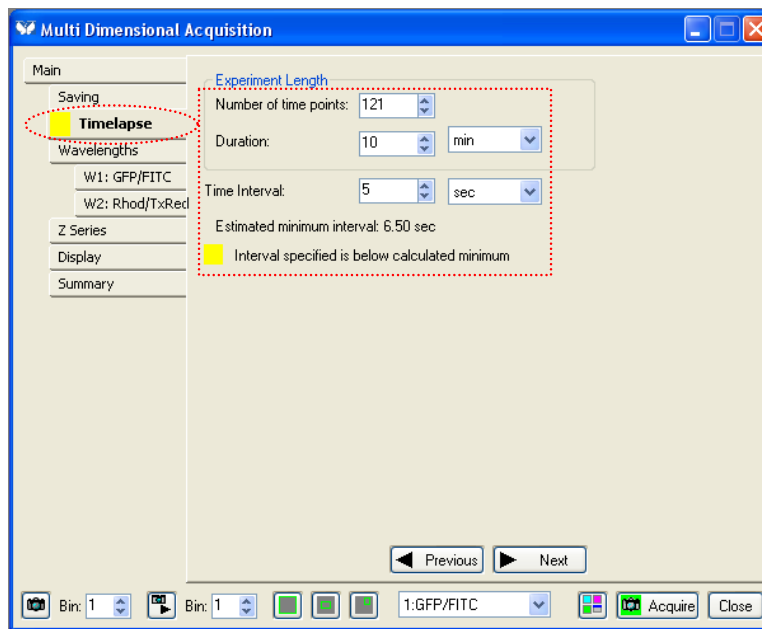
☞ Once your Z Series has been set up, click .

7) *Setting up your Timelapse*

☞ In the Main tab, select Timelapse.



☞ Select the Timelapse tab within the MDA.



☞ First, enter your desired time interval.

☞ Then, enter either your desired number of time points, or duration.

- **Note:** Be sure your time interval is greater than your acquisition time. Your acquisition time is shown below your timelapse parameters. For example: **Estimated minimum interval: 6.50 sec**. If your time interval is less than your total acquisition time, you will receive the following error: **Interval specified is below calculated minimum**

☞ Once your Timelapse has been set up, click **Acquire**.

8) System Shutdown

☞ Before you proceed with the shutdown sequence, please remember to back up all of your data and to clean the dry and oil immersion lenses.

☞ Sign onto the facility calendar and check for users after your session. If you don't see anything scheduled for less than 2 hours, proceed with the full shutdown sequence shown on the Shutdown poster. Otherwise, close the software and log off your account.

☞ If you do have to perform a full shutdown, please follow the shutdown poster steps in order.